



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
REGION 10
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October 11, 2006

Mr. Jim McKenna
Port of Portland & Co-Chairman, Lower Willamette Group
121 NW Everett
Portland, Oregon 97209

Mr. Robert Wyatt
Northwest Natural & Co-Chairman, Lower Willamette Group
220 Northwest Second Avenue
Portland, Oregon 97209

Re: Portland Harbor Superfund Site; Administrative Order on Consent for Remedial Investigation and Feasibility Study; Docket No. CERCLA-10-2001-0240. Round 3 Lamprey Ammocoete Toxicity Testing Field Sampling Plan

Dear Messrs. Wyatt and McKenna:

EPA has reviewed the Round 3 Lamprey Ammocoete Toxicity Testing Field Sampling Plan (Lamprey Toxicity FSP). This document was prepared on behalf of the Lower Willamette Group (LWG) by Windward Environmental and was received by EPA on September 29, 2006. EPA understands the collection of lamprey ammocoetes to be used for the toxicity testing needs to commence as soon as possible because the onset of significant precipitation events and declining surface water temperatures may make it difficult or impossible to collect the necessary tissue to perform the range finding tests described in the Lamprey Toxicity FSP. EPA comments are limited to comments related to the collection and holding of the lamprey ammocoetes. Comments on the range finding test are being withheld until the Quality Assurance Project Plan (QAPP) for the range finding testing has been received. This is to allow the lamprey ammocoete collection to begin as soon as possible.

EPA comments on the collection and holding of lamprey ammocoetes are provided below. Please confirm that the collection and holding will incorporate the changes outlined below.

General Comment

Section 1.1.1 of the Lamprey FSP describes a series of objectives for the Phase 1 lamprey ammocoete collection and testing effort. EPA does not believe that rigorous "methods" for the collection, transport and holding of the lamprey ammocoetes will be developed. Rather, one goal of the Phase 1 work will be to collect, transport and hold the ammocoetes for the Phase 1 range finding toxicity testing and to apply lessons learned regarding the collection, transportation and holding of lamprey ammocoetes for the Phase 2 toxicity testing.

Collection of Lamprey Ammocoetes

1. General: EPA assumes that the Siletz River and/or other watersheds targeted for either Phase 1 or Phase 2 lamprey ammocoete collection efforts as toxicologically clean reference/background samples. During the collection of lamprey ammocoetes for the toxicity testing, additional lamprey ammocoetes should be collected for chemical analysis. Lamprey ammocoetes collected as reference samples should be analyzed according to the protocols in the EPA approved Field Sampling Plan: Round 3 Sampling for Lamprey (*Lamperta* SP.) Ammocoete Tissue.
2. General: A representative sub-sample of the collected individuals should be archived for future reference and more detailed taxonomic identification if necessary.
3. Section 2.3.1, Page 9, Field Logs: Site conditions should be noted in the field logbook, including habitat, substrate type, water quality and density of ammocoetes within a specified collection area.
4. Section 2.5, Page 9, Schedule: It is unclear whether a target field water temperature of 10°C is critical to the success of the study. It is likely that stream flow may be a more critical factor governing the success of the lamprey ammocoete collection event. In either event, alternate locations should be identified to maximize the likelihood of a successful lamprey ammocoete collection event. Should productivity of the collection effort decline in response to lower temperatures and/or precipitation events, consultation with EPA is required to determine when to cease collection efforts.
5. Section 3.1, Page 10, Field Sampling, First Paragraph: The rationale for targeting “up to 800 ammocoetes” for collection is not provided. Further discussion is required to confirm the number of ammocoetes required for the Phase 1 work. This will be important to determine when to cease the Phase 1 ammocoete collection effort.
6. Section 3.1, Page 10, Field Sampling, Third Paragraph: It is unclear what is meant by the term “representative individuals.” In addition, the rationale for focusing on the “smallest” ammocoetes is unclear. For example, are smaller ammocoetes likely to be more sensitive to exposure to contaminants than are larger ammocoetes? Once collection efforts have begun, discussion with EPA is required to determine the size range to be transported to the laboratory for the Phase 1 work. EPA agrees that the ammocoetes submitted for Phase 1 testing should be of comparable size.

Holding of Lamprey Ammocoetes:

1. General: A key aspect of the toxicity testing program is the successful holding of the lamprey ammocoetes. Scientists from USGS, USFW, ODFW, the Siletz and others familiar with the holding of lamprey ammocoetes should be consulted to identify key factors and techniques associated with successful holding of lamprey ammocoetes.
2. Section 4.1, Page 12, Laboratory Holding: The health of the lamprey ammocoetes should be monitored closely during holding. EPA recommends maintaining careful records of the water conditions that the ammocoetes are being held in. Metrics for determining the health of the ammocoetes should be maintained. These may include regular weighing; monitoring of behavior such as swimming, burrowing, avoidance, or other responses; measuring respiration rate and mortality. EPA expects that modifications to the holding procedures may be required. For example, varying temperature or feeding in a consistent manner among different holding chambers, and varying the size of the holding chambers/density of fish. It is critical that any changes to the holding protocol be documented and summarized to ensure that Phase 2 is successful.

3. Section 4.1, Page 12, Laboratory Holding: The Lamprey Toxicity FSP states that water hardness will be soft (<50 mg/l as Ca CO₃). It is unclear how the water hardness was selected. For example, is the goal to match the hardness of the water from which the organisms were collected or to match the conditions of the Portland Harbor Study Area? Mean water hardness in the ISA is 30 mg/L (range approximately 25-35 mg/L for most samples). EPA recommends matching the hardness of the water from the collection area. Hardness may need to be varied to ensure successful holding of the ammocoetes. Other parameters (e.g., DO, pH) that may affect survival of the lamprey ammocoetes should be monitored and adjusted as necessary.
4. Section 4.1, Page 12, Laboratory Holding: The type and source of the laboratory exposure water should be specified. From the sediment toxicity studies conducted for the Portland Harbor RI/FS at NAS, EPA assumes that the water source will be dechlorinated City of Newport water; this assumption should be confirmed. In addition, EPA recommends transitioning the lamprey from Siletz River water to the laboratory water gradually in case there might be issues with acclimation to the different waters. Given that so little is known about animal husbandry with this species, such a gradual transition to the test waters may help maintain organism vigor.
5. Section 4.1, Page 12, Laboratory Holding: The Lamprey Toxicity FSP states “The water temperature in the holding aquaria will be 12° ± 2° C (Not to exceed 15° C).” The basis for selecting 12° C is unclear. Meeuwig et al. (2005) shows that among four temperature treatments (12°, 14°, 18°, and 22° C), lamprey at 18° C had the highest survival rate; although the only statistical difference was that lamprey at 22° C had a lower survival rate than lamprey maintained at the three other temperatures. Similarly, the temperature at which lamprey suffered the lowest rate of developmental abnormalities was 14° C, although the only statistical difference was that lamprey at 22° C had a lower rate of developmental abnormalities than lamprey maintained at the three other temperatures. Based on these data, the optimal temperature for maintaining ammocoetes in the lab would be between 14° and 18° C, not 12° C. This issue also is important because, in “cold-blooded” fish (and other organisms), toxicity of contaminants typically increases as temperature increases because of the effect of temperature on metabolic rate. Thus, lamprey ammocoetes may be more sensitive to contaminants at 16° - 18° C, than at 12° C.
6. Section 3.2, Page 11, Field Holding and Transportation, First Paragraph: It is unclear how was the substrate (sterile sand) in which the ammocoetes are transported was selected. Maintaining the natural conditions may be preferred during transport (assuming no unknown contamination in the source material).

EPA appreciates LWG's efforts to develop the Lamprey Toxicity FSP. Please acknowledge your acceptance of the above comments no later than close of business, October 13, 2006. Please contact Chip Humphrey at (503) 326-2678 or Eric Blischke (503) 326-4006 if you have any questions. All legal inquiries should be directed to Lori Cora at (206) 553-1115.

Sincerely,

Chip Humphrey
Eric Blischke
Remedial Project Managers

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